

A NEW SPECIES OF THE GENUS *POLYSTOMA* (MONOGENEA, POLYSTOMATIDAE) PARASITIC IN TREE FOG *PHILAUTUS PALPEBRALIS* SMITH

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Abstract New taxon was proposed for *Polystoma zuoi* sp. nov. from the urinary bladder of the host *Philautus palpebralis* Smith, 1924. The hosts were obtained at Pingbian County of Yunnan Province. Out of 73 frogs, 4 male hosts were infected with the new parasites with prevalence of 5.5%. Mean total body length of the new species is about 2 716 μm ; mean total body width is about 1 280 μm . It is very similar to *P. carvirostris* which parasitic in *Rhacophorus carvirostris* at the same habitat by morphological feature of intestinal caecum. Otherwise the new species is distinguished from *P. carvirostris* by a combination of characters, including the parasite size, shape and size of hamuli and haptor. The new species is smaller than *P. carvirostris* but the size of haptor and the hamuli are distinctly larger completely. The two polystomatids

have 8 skeletal genital spines, but it is rather different at the shape. For *P. carvirostris*, genital spines are finer and fasciations dispersedly. Genital spines of new species present recurved basal and fine distal, appearance such as coronary. Phylogenetic relationships of *P. zuoi* with *Polystoma*, supplemented with molecular divergences estimated from 18S rRNA genes sequences indicate that they are well separated from their closest relatives, i. e. *P. carvirostris* from *P. zuoi*. This molecular research suggest that although the new species shares various morphological features with *P. carvirostris*, the phylogenetic-position of the new species is a new taxon, namely after Zuo Yangxian, professor at parasitological of Yunnan University. Specimens were conserved in the School of Life Sciences of Yunnan Normal University.

Key words Polystomatidae, *Polystoma*, *Philautus palpebralis*, new species.

白颊小树蛙寄生多盘虫属单殖吸虫一新种

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摘要 从云南省屏边县采集的白颊小树蛙 *Philautus palpebralis* Smith, 1924 膀胱内检获多盘虫属 1 新种, 命名为左氏多盘吸虫 *Polystoma zuoi* sp. nov.。73 只白颊小树蛙中有 4 只雄蛙感染该种吸虫, 感染率约为 5.5%。新种虫体全长平均为 2 716 μm , 体宽平均为 1 280 μm 。与肠管形态相似的锯腿树蛙多盘吸虫 *P. carvirostris* 比较, 两者体型、后吸器、大锚钩及生殖棘存在显著区别。锯腿树蛙多盘吸虫体长平均为 5 070 μm , 明显大于本种。而本种的后吸器和大锚钩则比锯腿树蛙多盘吸虫的大。两者生殖棘均为 8 枚, 但锯腿树蛙多盘吸虫的生殖棘较细, 分散, 成簇状, 而本种的生殖棘基部较宽并向外卷曲, 尖端较细, 聚拢为冠状。基于 18S rRNA 基因序列的补充研究表明, 本种与锯腿树蛙多盘吸虫, 应为不同的物种。模式标本保存在云南师范大学生命科学学院。

关键词 多盘虫科, 多盘虫属, 白颊小树蛙, 新种。

中图分类号 Q959.153

多盘虫属 *Polystoma* Zeder, 1800 隶属于扁形动物门 Platyhelminthes 单殖吸虫纲 Monogenea 多盘虫目

Polystomatidea 多盘虫科 Polystomatidae。多盘虫属因后吸器具有 6 个吸盘和 1 对几丁质大锚钩而得名。

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至2012年,全球记录多盘虫科单殖吸虫22属约200余种,其中多盘虫属65种(Yildirimhan *et al.*, 2012)。我国记录多盘虫属5种(Wu *et al.*, 2000; Fan *et al.*, 2004; Fan *et al.*, 2008; Meng *et al.*, 2010; Gao *et al.*, 2012)。多盘虫属属内新种认定的主要分类学性状包括后吸器、大锚钩、肠管分支和生殖系统等形态结构。Combes (1966, 1968), Tinsley (1973, 1974), Euzet (1974) 和 Du Preez 等 (1987, 1992, 1993, 1997) 通过研究欧洲和非洲多盘虫属吸虫的生活史和实验感染指出多盘虫属具有严格的宿主特异性 (host-specificity), 其宿主的分类学地位可作为区分属内种的重要依据 (Combes *et al.*, 1979, 1995; Du Preez *et al.*, 1997, 2007, 2011; Aisien *et al.*, 2011; Yildirimhan *et al.*, 2012)。同时鉴于形态学分类特征容易受到标本处理方法以及寄生虫的年龄、性成熟程度等因素的影响,在单殖吸虫新物种的描述研究中需要补充其它研究方法 (Ding *et al.*, 2005; Fan *et al.*, 2007)。例如通过分子生物学研究方法,比对物种之间18S rRNA、ITS1 和28S rRNA 基因序列等的遗传学特征,确定单殖吸虫的分类地位,斧正形态学分类的结果 (Jia *et al.*, 2010; Aisien *et al.*, 2011)。

本文记述了寄生于白颊小树蛙 *Philautus palpebralis* 膀胱内多盘虫属未记录吸虫的主要形态学特征,对其形态学分类性状进行了数值测量;采用分子生物学方法进行补充研究,测试分析了基于18S rRNA 基因序列片段近似物种之间的遗传距离 (P 模式遗传距离),重建了系统发育树。研究表明,形态学分类的结果与分子生物学研究的结果互为印证,该种吸虫为科学上未记录的新种,命名为左氏多盘吸虫 *Polystoma zuoi* sp. nov.。模式标本保存于云南师范大学生命科学学院动物学教研室。

左氏多盘吸虫,新种 *Polystoma zuoi* sp. nov. (图2~7)

宿主:白颊小树蛙 *Philautus palpebralis*。

寄生部位:膀胱。

采集时间及地点 2011年5月26日,云南省屏边县 (22°57'N, 103°42'E; 海拔1607 m)。采集地为低洼水塘,四周为乔木和灌木丛。正模编号 Pal2011052601, 副模编号 Pal2011052602 和 Pal2011052603, 模式标本保存于云南师范大学生命科学学院动物学教研室。

宿主鉴定依据《云南两栖类志》和《中国两栖动物检索及图解》(Yang, 1991; Fei *et al.*, 2005)。

采集白颊小树蛙73只 (68♂♂、5♀♀), 其中4只 (♂♂) 感染,自然感染率约为5.5%,感染强

度为1~3枚,平均感染强度为1.5,感染密度为0.082。

对3枚封片标本进行了形态学分类研究,测量数值 (表1) (文中量度单位以微米 μm 记)。虫体 (图1~3) 全长1624~3533 (平均2716), 宽1202~1904 (平均1280)。后吸器大小为632×831~924×1317 (平均701×1063)。吸盘直径209~311 (平均251)。中央大锚钩较明显,基部较宽,有少量凹痕,无明显的裂纹,大锚钩爪尖较细长,略弯曲 (图4)。内外突分化不明显,外柄X值185~307 (平均240), 内柄Y值173~265 (平均205), X/Y 约为1.07~1.26 (平均1.16)。

咽球形明显,之后为两分支的肠,肠管外侧无分支,内侧形成多个分支。中后部肠管的内侧分支联接形成肠管联合3~4个,近生殖腔的肠管内侧分支未见过体中线的肠管联合。两侧肠管于体后端合并形成联合肠管,并向后延伸,进入后吸器,进入后吸器的肠管未见明显的盲肠突起。

卵巢较明显,椭圆形,中部有缢缩,位于生殖腔一侧,卵巢长为329~400 (平均369)。子宫内未见虫卵。阴道孔位于卵巢偏下方的两侧。生殖棘8枚,基部较宽并向外卷曲,尖端较细窄,尖端合拢形如冠状 (图5)。

采用分子生物学研究方法 (Jia *et al.*, 2010), 测定了本种的核糖体18S rRNA 基因序列,采用Clustal X 软件对其18S rRNA 基因的1104个位点进行了比对。结果表明:本种18S rRNA 基因序列共有保守位点917个,变异位点183个,包括146个简约信息位点和37个变异位点。A、T、G、C 碱基的平均含量为24.4%、28.3%、26.6%、20.7%,且A+T的含量高于G+C的含量。转换/颠换比率为1.63。从GenBank 获得近似种18S rRNA 基因序列,用MEGA 5.0分析了变异信息位点,计算了该片段两个物种之间的遗传距离 (P 模式遗传距离)。结果显示,本种与锯腿树蛙多盘吸虫 *P. carvirostris*、新平多盘吸虫 *P. ximpingensis* 的P模式遗传距离分别为0.013和0.029;锯腿树蛙多盘吸虫 *P. carvirostris* 与新平多盘吸虫 *P. ximpingensis* 的P模式遗传距离为0.02。本种、锯腿树蛙多盘吸虫和新平多盘吸虫可视为不同的分类单元。基于18S rRNA 基因序列重建系统发育树 (ML, 图6) 表明,本种与多盘虫属其它物种聚为一个 clade 并位于多盘虫属 clade 的基部,可视为多盘虫属较为原始的物种。本种与锯腿树蛙多盘吸虫聚为一支,可能说明两者亲缘关系最近。

讨论 本种与锯腿树蛙多盘吸虫 *P. carvirostris* 肠管形态结构较为相似,两者的内侧肠管都形成多个

表 1 左氏多盘吸虫形态结构数据与多盘虫属纪录种的比较 (量度单位: μm)

Table 1. Measurements of *Polystoma zuoi* sp. nov. compare with recorded species in genus *Polystoma*.

	屏边多盘吸虫 <i>P. pingbianensis</i>	斑腿树蛙多盘 吸虫 <i>P. leucomystax</i>	无声囊泛树蛙 多盘虫 <i>P. mutus</i>	<i>P. testimagna</i>	<i>P. gallieni</i>	新平多盘吸虫 <i>P. xingpingensis</i>	锯腿树蛙 多盘吸虫 <i>P. caruirostris</i>	左氏多盘吸虫, 新种 <i>Polystoma zuoi</i> sp. nov.
宿主 Host	杜氏泛树蛙 <i>Polydactylus daigritzi</i>	斑腿树蛙 <i>Rhasophorus leucomystax</i>	无声囊泛树蛙 <i>P. mutus</i>	<i>Strangophus f. fasciatus</i>	<i>Hyla meridionalis</i>	<i>H. a. annectans</i>	锯腿树蛙 <i>R. caruirostris</i>	白颊小树蛙 <i>Philautus palpebralis</i>
体长 Body length	9 430	7 568	7 338	7 850	5 500	2 967	5 070	2 716 (1 624 ~ 3 533)
体最宽处 Greatest width	1 990	2 732	3 063	2 580	1 100	1 187	1 130	1 280 (1 202 ~ 1 904)
过阴道处宽 Width at vaginae	15 72	-	-	1 680	-	904	830	-
后吸器长 Haptor length	869	824	913	2 050	-	801	680	701 (632 ~ 924)
后吸器宽 Haptor width	784	1693	1 650	2 860	-	1 211	890	1 063 (831 ~ 1 317)
咽长 Pharynx length	253.2	252	245	293	-	199	-	164
咽宽 Pharynx width	229.2	216	235	294	-	154	-	151
卵长 Egg length	306.6	-	-	228	180	251	-	-
卵宽 Egg width	164.6	-	-	169	100	91	-	-
卵巢长 Ovary length	711.5	608	588	836	-	474	497	369 (329 ~ 400)
吸盘直径 Sucker diameter	434.4	289	310	553	-	275	259	251 (209 ~ 311)
X/Y	1.06	-	1.17	1.24	-	1.27	1.17	1.16 (1.07 ~ 1.26)

表 2 采用的多盘吸虫及其宿主、来源地、18S rRNA 基因序列的 GenBank 登录号、进化支及序列长度

Table 2. List of polystomatids cited in the present study, including host species, family, geographical origin, GenBank accession numbers length and clade of 18S rRNA gene sequence.

Parasite species (number of sample)	Host species	Host family	Geographical origin	Accession number of 18S	Clade	Sequence length (bp)
<i>Polystoma davieholki</i>	<i>Psychadena anchietae</i>	Ranidae	South Africa	AM051069	African	2 124
<i>Polystoma occipitalis</i>	<i>Hemisus marmoratus</i>	Ranidae	Ivory Coast	AM051075	African	2 095
<i>Polystoma gullieni</i>	<i>Hyla meridionalis</i>	Hylidae	France	AM051070	Eurasian	2 123
<i>Polystoma pelobatis</i>	<i>Pelobates cultripes</i>	Pelobatidae	France	AM051076	Eurasian	2 122
<i>Polystoma marmorati</i>	<i>Hyperolius marmoratus</i>	Hyperoliidae	South Africa	AM051073	African	2 095
<i>Polystoma integerrimum</i>	<i>Rana temporaria</i>	Ranidae	France	AM051071	Eurasian	2 094
<i>Polystoma nearcticum</i>	<i>Hyla versicolor</i>	Hylidae	USA	AM051074	American	2 118
<i>Polystoma lophoromani</i>	<i>Phrynobyas venulosa</i>	Hylidae	Paraguay	AM051072	American	2 086
<i>Polystoma cavierei</i>	<i>Physalaemus cavierei</i>	Leptodactylidae	Paraguay	AM051068	American	2 092
<i>Polystoma caruirostris</i>	<i>Rhacophorus caruirostris</i>	Rhacophoridae	China	EU734835	Eurasian	1 517
<i>Polystoma</i> sp.	<i>Rana chaochiaoensis</i>	Ranidae	China	EU734834	Eurasian	1 522
<i>Polystoma xingmogensis</i>	<i>Hyla a. amictans</i>	Ranidae	China	EU979386	Eurasian	1 520
<i>Wetapiostoma abnae</i>	<i>Bufo margaritifera</i>	Bufonidae	French Guiana	AM051081	American	2 092
<i>Neodiplorhynchus scaphiopi</i>	<i>Spea bombyfrons</i>	Pelobatidae	USA	AM051067	outgroup	1 994
<i>Pseudodiplorhynchus americanus</i>	<i>Scaphiopus couchii</i>	Pelobatidae	USA	AM051079	outgroup	2 020
<i>Protopolystoma occidentalis</i>	<i>Xenopus muelleri</i>	Pipidae	Togo	AM051077	outgroup	2 024
<i>Protopolystoma xenopodis</i>	<i>Xenopus laevis</i>	Pipidae	South Africa	AM051078	outgroup	1 991
<i>Polystoma zuoi</i> sp. nov.	<i>Philautus palpebralis</i>	Rhacophoridae	China		Eurasian	1 102

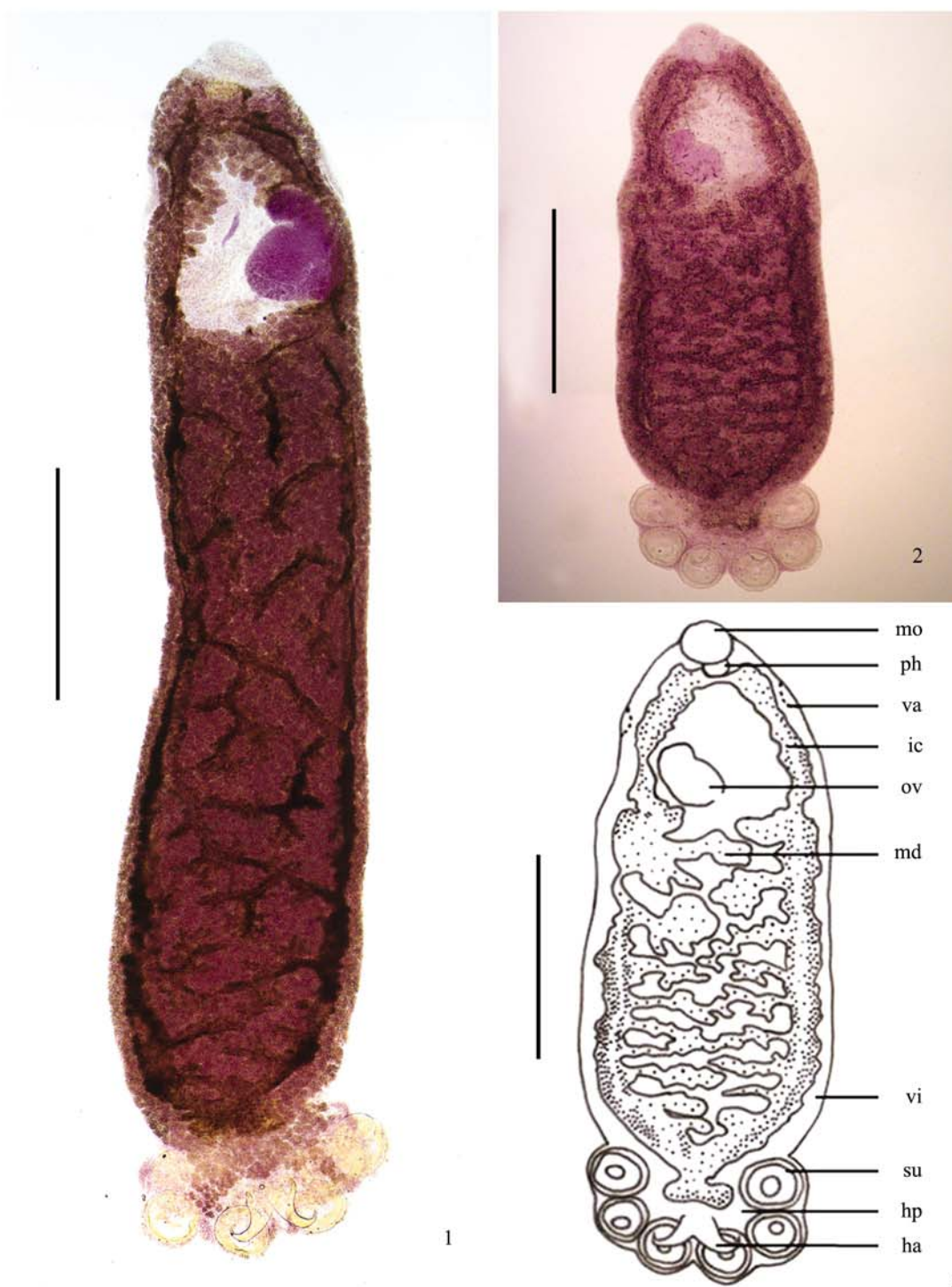


图1 锯腿树蛙多盘吸虫 *P. carvirostris* 图2~3 左氏多盘吸虫, 新种 *Polystoma zuoi* sp. nov.

1~3. 腹面观 (ventral view) Abbreviations: ha. Hamulus. hp. Haptor. ic. Intestinal canal. md. Medial diverticula. mo. Mouth. ov. Ovary. ph. Pharynx. su. Sucker. vg. Vagina protrusion. vi. Vitelline distribution. 比例尺 (scale bars) = 1 mm

侧分支。但本种近后吸器处的肠管侧分支连接形成多个肠管联合, 近生殖腔处基本没有肠管联合。本种与锯腿树蛙多盘吸虫的生殖棘都为8枚, 后者生殖棘较细, 分散, 成簇状, 而前者生殖棘基部较宽并向外卷曲, 尖端较细, 聚拢为冠状。本种的体长和体

最宽处分别为2 716 和1 280, 锯腿树蛙多盘吸虫的体长和体最宽处分别为5 070 和1 130, 本种后吸器大小平均701×1 063, 锯腿树蛙多盘吸虫后吸器大小平均为680×890, 可见在体型明显小于锯腿树蛙多盘吸虫的情况下, 本种的后吸器和大锚钩大于锯腿

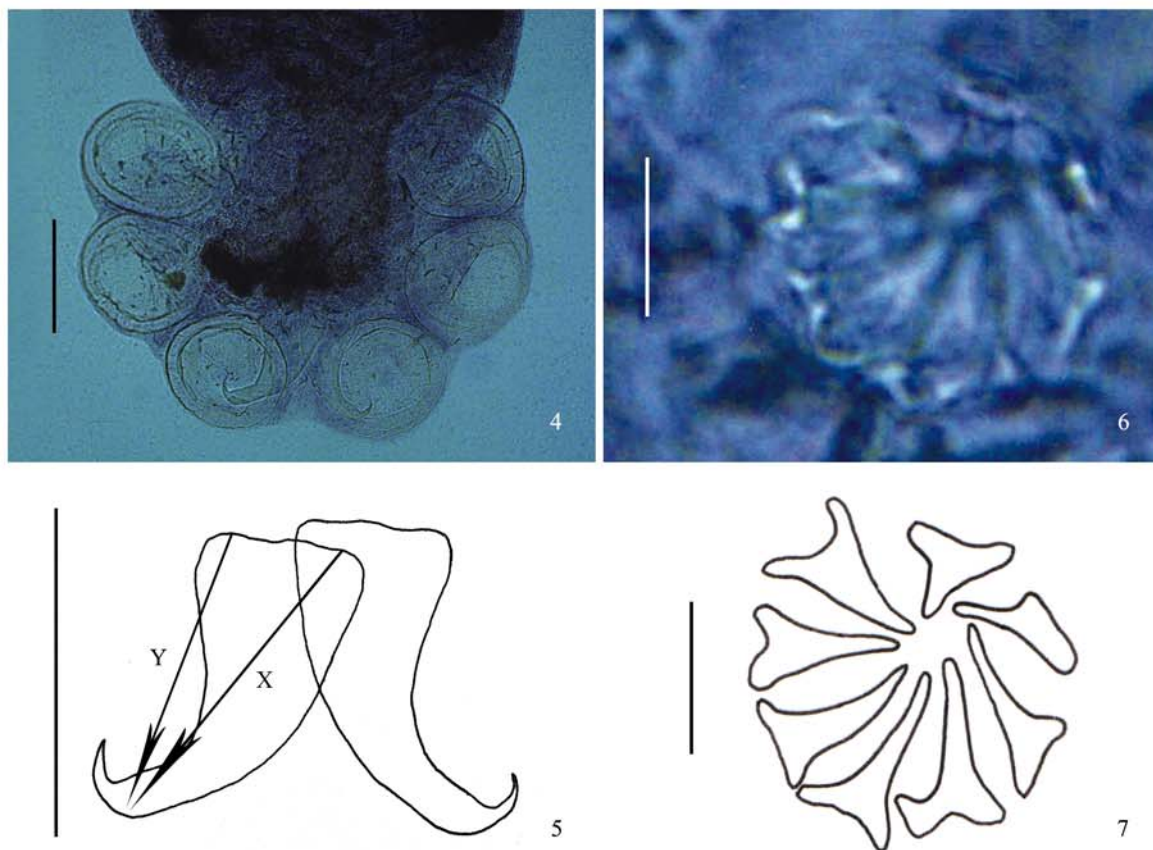


图4~7 左氏多盘吸虫, 新种 *Polystoma zuoi* sp. nov.

4~5. 后吸器及中央大锚钩 (haptor, sucker and hamuli from holotype) X. Distance from hook to tip of handle.

Y. Distance from hook to tip of guard. 6~7. 生殖棘 (genital spines) 比例尺 (scale bars): 4~5 = 200 μm , 6~7 = 20 μm

树蛙多盘吸虫。基于 18S rRNA 基因序列的比对研究, 本种与锯腿树蛙多盘吸虫的 P 模式遗传距离为 0.013, 二者可视为不同的分类单元。

通过上述形态学分类研究和基于 18S rRNA 基

因部分序列的分子生物学研究, 认为该种多盘吸虫为科学上的未记录种, 命名为左氏多盘吸虫 *Polystoma zuoi* sp. nov.。

词源: 新种种名源自云南大学左仰贤教授之名。

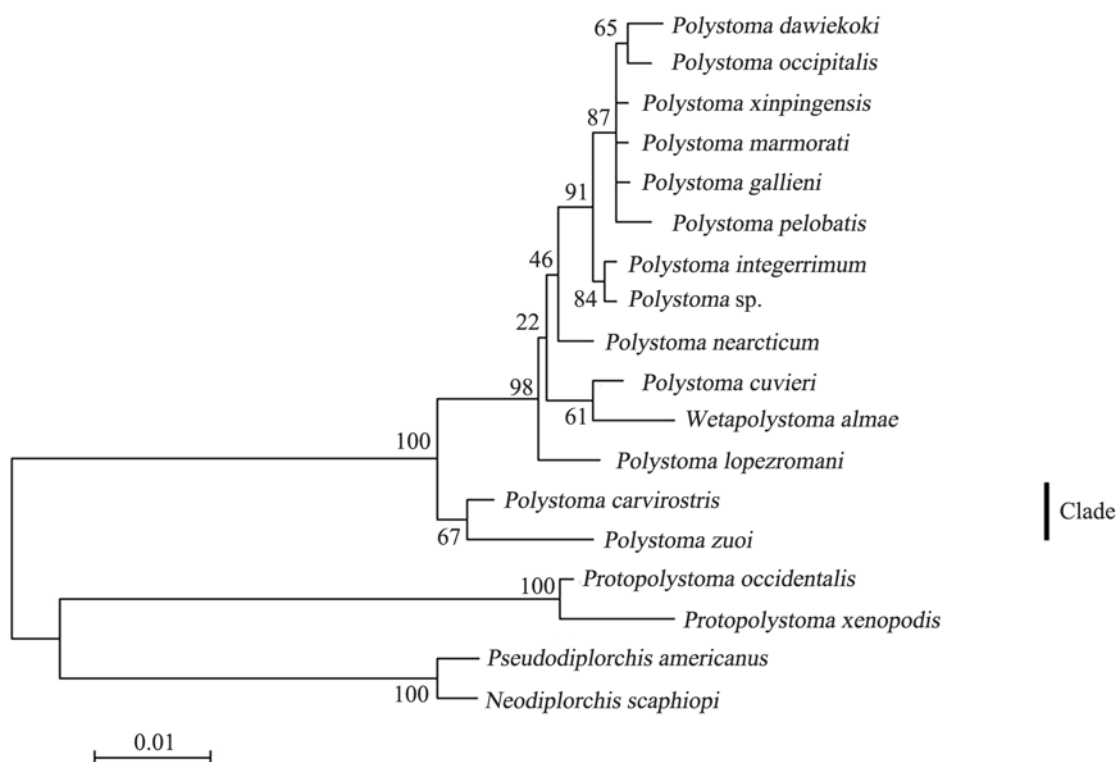


图 8 基于 18S rRNA 基因部分序列构建的 ML 树, 各分支上的数字为 Bootstrap 1 000 个循环自举检验值

Fig. 8. ML tree based on partial sequence of 18S rRNA gene with confident values of Bootstrap 1 000 indicated above each branch.

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